REVIEW

Problems in the biological monitoring of chromium(VI) exposed individuals

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The usefulness of currently available techniques for the biological monitoring of chromium(VI) exposed individuals is reviewed. Chromium levels in body fluids, such as urine and blood plasma, are reliable markers of exposure to chromium in oxidation states (VI) and (III) and provide a measure of the internalized dose of chromium. These markers are sufficiently sensitive to be useful in most occupational settings encountered today. In contrast, the majority of cytogenetic surveillance studies among chromium platers, ferrochromium workers and stainless steel welders using the manual metal arc (MMA) method have yielded negative or inconclusive results. As a marker for genotoxicity, the number of sister chromatid exchanges in blood lymphocytes proved to be relatively insensitive towards exposure to chromium(VI). There were however significant increases in rare chromosome aberrations among MMA stainless steel welders, although the reported levels of all aberrations combined were similar to those observed among control groups of many other studies. The relative lack of success of cytogenetic surveillance studies using blood lymphocytes is surprising in view of the strong genotoxicity of chromium(VI). A possible explanation comes from recent studies which showed that the differences in chromium lymphocyte levels between exposed and controls were disproportionately small. Another factor which complicates attempts to correlate genotoxic effects in lymphocytes with the processes giving rise to cancers of the respiratory system is the toxicokinetics of inhaled chromium(VI). Only small fractions of the total inhaled dose are distributed in the body while the bulk of chromium(VI) deposited in the lungs remains there for very long periods of time. The vast majority of lymphocytes will therefore come into contact with chromium(VI) not while travelling through the supporting tissues of the lungs but during their migration through the blood. There they take up chromium(VI) that has leached from the lungs. Blood lymphocytes therefore seem to be inappropriate for the monitoring of the biologically effective dose, and of early biological effects arising from exposure to chromium(VI). Thus there is an urgent need to develop techniques which would allow the non-invasive monitoring of internalized doses of chromium in the lung.

Keywords: *chromium, biomonitoring, lymphocytes, cytogenetic surveillance studies.*

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Introduction

Exposure to chromium(VI) compounds by inhalation is of concern in a variety of industries including the primary production of chromates, chromium plating, chromate pigment manufacture and the welding of the stainless steel. Stainless steel welding, and in particular the manual metal arc (MMA) welding method, may well be the most common source of occupational exposure to chromium(VI), given the fact that there are millions of stainless steel welders worldwide (NIOSH 1977, IARC 1990). With many of the potentially hazardous workplaces identified, the major concern today is to establish a relationship between exposure and cancer risks and to define safe levels of exposure on a sound toxicological basis. In this context, the biological monitoring of exposed individuals is of great importance. During the last decade, a number of surveillance techniques have been developed which allow the monitoring of internal exposure to chromium(VI) by analysing body fluids, e.g. blood, plasma, and urine. The monitoring of the possible genotoxic effects of chromium(VI) was carried out by assessing chromosomal aberrations, sister-chromatid exchanges (SCEs) and DNA damage in easily accessible tissue, usually blood lymphocytes. Whilst exposure to chromium(VI) at levels well below 50 $\mu g\ m^{-3}$ (the occupational exposure limit in a number of countries) can be reliably verified by determining chromium concentrations in urine or plasma, opinions are divided as to the usefulness of cytogenetic surveillance techniques. How effective are currently available biological monitoring techniques for the screening of internal exposure to chromium(VI) and for the assessment of genotoxic effects?

In the biomonitoring of chemical carcinogens it is useful to distinguish between the monitoring of an internalized dose, a biologically effective dose, and an early biological effect (Wogan 1992). The level of carcinogens and their metabolites in body fluids can be regarded as a measure of the internalized carcinogen dose. Although useful in confirming that exposure has indeed occurred, measures of an internalized dose *per se* do not give any indications of the potential cancer risks involved. More closely related to cancer risks are measures of the biologically effective dose, e.g. the level of DNA damage or DNA adduct formation in certain tissues. Genetic alterations such as chromosome aberrations, SCEs and gene mutations are early biological effects in carcinogenesis. The monitoring of these markers is carried out using nucleated cells in easily accessible tissue, usually blood lymphocytes. The evaluation of genetic effects in peripheral blood lymphocytes is based on the assumption that these cells are valid surrogates for the changes taking place in tissues where neoplasms may eventually develop. However, many other cells of the body show DNA damage and even cytogenetic effects as a result of interactions with carcinogenic chemicals, yet never develop into cancer cells.

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Monitoring internal exposure to chromium(VI): the analysis of chromium levels in body fluids

A number of investigations have helped to establish the relationship between airborne chromium and the levels of chromium in blood, plasma and urine (Gylseth *et al.* 1977, Tola *et al.* 1977, Mutti *et al.* 1979, Fleischer and Schaller 1980, Kalliomäki *et al.* 1981, Lewalter *et al.* 1985, Angerer *et al.* 1987). Urinary chromium can be regarded as a reliable marker of internal chromium exposure, and is sufficiently sensitive for biological monitoring at exposure levels well below the occupational limits in a number of industrialized countries. Table 1 summarizes the urinary chromium levels observed in selected studies of occupationally exposed and non-exposed individuals as well as diabetics and beer drinkers.

On the basis of urinary chromium alone it is not possible to distinguish whether exposure to chromium(VI) or chromium(III) has occurred. Only chromium in erythrocytes is diagnostic for internal exposure to chromium(VI). This is because the chromate anion, unlike chromium(III) complexes, is able to cross cell membranes of red blood cells via anion carrier proteins (Gray and Sterling 1950, Kortenkamp *et al.* 1987). However, using this technique the advantage of diagnostic specificity is achieved at the expense of diagnostic sensitivity. In a study among stainless steel welders airborne chromium(VI) at levels of around 100 μg m^{-3} did not give rise to elevated concentrations in the red blood cells of about 50% of the exposed workers (Angerer *et al.* 1987). Chromium levels in plasma and urine were increased at much lower exposures. The measurement of chromium in plasma therefore opens the possibility for monitoring a wider range of exposure levels likely to be encountered in occupational settings, albeit at the expense of diagnostic specificity for exposure to chromium(VI).

Because of its sensitivity and convenience of use, the determination of chromium in urine is by far the most widely used method for the monitoring of internal chromium exposure. However, urinary chromium levels are affected by variations in renal function. These can be corrected for by relating urinary chromium concentrations to the amount of creatinine present in the urine (Araki and Aono 1989). The correlations between urinary and plasma chromium levels are excellent, so that one monitoring method can conveniently be substituted for the other (Angerer *et al.* 1987, Strindsklev *et al.* 1993).

A factor which has to be taken into consideration during the design of monitoring programmes is that smokers usually show higher urinary chromium levels than non-smokers (Kalliomäki *et al.* 1981, Strindsklev *et al.* 1993) (Table 1). Bukowski *et al.* (1991) have conducted a study aimed at establishing further variables which might confound urinary chromium levels. They observed that lack of exercise, drinking beer and diabetes all lead to slightly elevated urinary chromium levels. Erythrocyte chromium levels were not affected by either of these factors.

Mean Cr(VI) in air (μg m^{-3})	Group	Mean urinary Cr (μg g^{-1} creatinine)	Reference
13.8	SS, smokers	14.2	Strindsklev *et al.* 1993
13.5	SS, non-smokers	5.3	
na	SS, smokers	16	Kallionmäki *et al.* 1981
na	SS, non-smokers	8.7	
1–55	Cr(VI) production	6	Gao *et al.* 1994
0	Non-exposed	0.76	
0	Diabetics	1.38	Bukowski *et al.* 1991
	Referents	0.58	
0	Beer drinkers	0.67	
	Referents	0.49	

Table 1. Urinary chromium levels among occupationally exposed and non-exposed individuals.

Key: na, not analysed; SS, stainless steel welders (MMA).

Chromium(VI) effect monitoring: human cytogenetic surveillance studies using blood lymphocytes

The majority of cytogenetic surveillance studies analysing chromosome aberrations and SCEs in lymphocytes of chromium(VI) exposed workers carried out in the 1980s and 1990s provide detailed information on measures of external and internal exposure to chromium. It is therefore possible to evaluate the relative sensitivity of markers of early biological effects and of markers of internal exposure. Chromium(VI) effect monitoring has focused on chromium platers, ferrochromium steel production workers and stainless steel welders.

Studies among chromium platers and ferrochromium steel production workers

Chromium platers are exposed to mists of chromium(VI) trioxide which are generated during electrolysis in plating baths containing chromium trioxide, sulphuric acid and various organic additives. The mists are formed when bubbles of oxygen and hydrogen arise from the electrodes and burst at the liquid surface of the plating bath. The use of surfactants or floating balls, combined with local exhausts, can substantially lower exposure to chromium(VI) trioxide. One step in the production of ferrochromium steel involves the electrothermal reduction of chromite ore with coke in furnaces. Workers near these furnaces are exposed to fumes containing chromium(VI) trioxide.

A compilation of cytogenetic studies among chromium platers and ferrochromium workers is shown in Table 2. In most of these studies urinary chromium was chosen as the marker of internal exposure. In order to enable comparisons to be made between studies, the levels of urinary chromium were expressed in relation to urinary creatinine; where necessary, conversions were made assuming 1.5 mg creatinine l^{-1}.

An interesting trend in Table 2 is the absence of marked differences in the numbers of chromosome aberrations or SCEs between exposed and control. The majority of the studies failed to demonstrate any statistically significant increases. The elevated SCE frequencies seen by Sarto *et al.* (1982) among hard platers remained apparent only in younger workers, when the data were analysed by age. There was a correlation between SCE frequency and both age and urinary chromium levels, with

Study population	Level and duration of exposure	Chromosome aberrations: Group	N	Ab+ gaps (%)	SCE/cell	Internal exposure (urinary Cr, μg g^{-1} crea)	Author's conclusion	Reference
Platers	na, 6 years	Exp	21	2.8	8.08	7–11	Positive	Sarto *et al.* 1982
		EC	35	1.7	6.6	2		
Platers	na, 0.5–30 years	Exp	24	na	9	8.7	Negative	Nagaya 1986
		MC	24	na	8.9			
Platers	27 μg m^{-3}	Exp I	7	na	6.9	16	Positive	Choi *et al.* 1987
	8 μg m^{-3}	Exp II	25	na	5.4	10.1		
	0	MC	15	na	3.6	na		
Ferrochromium workers	na	Exp	14	4.29	na	na	Negative	Sbrana *et al.* 1990
		MC	14	2.81				
Platers	na	Exp	12	4.29	7.8	17.9	Negative	Nagaya *et al.* 1991

Table 2. Cytogenetic surveillance studies among chromium platers and ferrochromium workers.

Key: *N*, number of subjects; Ab, aberrations; na, not analysed; crea, creatinine; Exp, exposed; EC, external controls; MC, matched controls.

higher frequencies occurring in young workers with high internal exposure to chromium. The significant increase in the number of chromosome aberrations in hard platers was only poorly correlated with urinary chromium levels. Choi *et al.* (1987) found elevated SCE frequencies in their groups of platers and chromium surface treatment workers, however the number of SCEs in their group of exposed workers was frequently observed in unexposed controls of many other cytogenetic surveillance studies. Ashby and Richardson (1985) reported the mean value of SCEs in control populations of 47 cytogenetic studies as 8.12±1.82 per cell, well above the level of 6.9±1.8 observed among platers or 3.6±1.5 among controls in Choi's study. Exposure to chromium was readily detected as elevated urinary chromium levels in all of the studies.

Studies of stainless steel welders (manual metal arc method)

Of the three methods of stainless steel welding, manual metal arc (MMA), metal inert gas (MIG) and tungsten inert gas (TIG), the MMA method produces welding fumes containing the largest amounts of chromium(VI). The total fume concentration in the breathing zone of MMA welders may be as high as 100 mg m^{-3}, with the level of chromium(VI) reaching 4 mg m^{-3} in extreme cases (Kalliomäki *et al.* 1981).

The results of the cytogenetic studies among MMA welders (Table 3) confirm some of the trends which became apparent in the plating studies. Again, urinary chromium proved to be a reliable marker of internal exposure even at relatively low levels of airborne chromium(VI). However, the frequency of SCEs in welders was surprisingly insensitive to chromium(VI) exposure at these levels. An increase in the number of SCEs was not observed in any of the studies except that of Koshi *et al.* (1984). The elevated frequency of SCEs reported by this group became detectable only after the outcome of three separate surveys had been pooled. In contrast, all the reports published after 1990 showed a tendency for SCEs to be slightly lower in MMA stainless steel welders than in unexposed controls. This effect seems to be obscured by smoking (see the data by Husgafvel-Purisainen *et al.* 1982), but is quite prominent among non-smokers (Popp *et al.* 1991, Knudsen *et al.* 1992, Jelmert *et al.* 1994), and in the study by Popp *et al.* (1991), even among smokers.

A slightly different picture emerges when chromosome aberrations are considered. While Husgafvel-Purisainen *et al.* (1982) and Littorin *et al.* (1983) failed to observe any significant changes in the number of aberrations, elevated frequencies were seen by Koshi *et al.* (1984), Knudsen *et al.* (1992) and Jelmert *et al.* (1994). Considering that small differences in effect require larger sample sizes to demonstrate statistical significance, the failure by Husgafvel-Purisainen *et al.* (1982) and Littorin *et al.* (1983) to detect aberrations is likely to be due to the relatively small study populations.

It is alarming that Koshi *et al.* (1984) and Knudsen *et al.* (1992) observed a statistically significant increase of rare aberrations such as dicentric chromosomes, translocations, minutes and rings among MMA welders. Knudsen *et al.* (1992) reported that these effects were only detected among non-smokers. In discussing this phenomenon, Knudsen *et al.* (1992) emphasized that these are severe genetic effects which require two independent damaging events to be expressed. A similar trend can be seen in the data for non-smoking MMA welders presented by Jelmert *et al.* (1994). The effects observed in the last two studies would have remained undetected had the study populations not been stratified according to smoking status.

The results of these investigations clearly force the conclusion that there is evidence for genotoxic effects among stainless steel welders using the MMA method. It is however important to evaluate these data in the context of other cytogenetic surveillance studies. The frequencies of aberrations detected among welders are similar to those observed in unexposed controls of many studies, which is about 1.42±0.96% of aberrant cells (excluding gaps) (Ashby and Richardson 1985). In view of the strong genotoxicity of chromium(VI) compounds (IARC 1990) it is surprising that the

Study population	Level and duration of exposure	Chromosome aberrations: Group	N	Ab + gaps (%)	SCE/cell	Internal exposure (urinary Cr, μg g^{-1} crea)	Author's conclusion	Reference
SS, MMA	55 μg m^{-3}, 19 years	Exp	24	4.1	11	20.8	Negative	Littorin *et al.* 1983
		EC	24	4.6	12	0.66		
SS, MMA	na	Exp, s	9	3.1	10.8	6.7–51	Negative	Husgafvel-Purisainen *et al.* 1982
		MC, s	10	1.9	10.5	na		
		Exp, ns	12	1.7	8.2	6.7–51		
		Mc, ns	9	2.4	8.9	na		
SS, MMA and MIG	na	Exp	97	**4.7**	**8.8**	6.5	Positive	Koshi *et al.* 1984
	na	IC	33	3.2	8.11	2.73		
		Group	N	Ab excluding gaps (%)				
SS, MMA and MIG	na, 8 years	Exp, s	17	na	6.61	18.9	Negative	Popp *et al.* 1991
		IC, s	7	na	7.34	na		
		Exp, ns	22	na	5.93	18.9		
		IC, ns	11	na	6.57	na		
SS, MMA and TIG	4.9±11.8 μg m^{-3}	Exp, s	32	2.25	7.7	2.08	Positive for Ab in ns, negative for SCE	Knudsen *et al.* 1992
		MC, s	50	1.85	8.1	0.35		
		Exp, ns	28	**2.38**	6.7	2.08		
		MC, ns	25	1.10	7.4	0.35		
		Group	N	Chromatid break (%)				
SS, MMA	34.7 μg m^{-3}, range 0.6–252 μg m^{-3}, 0–20 years	Exp, s	22	1.4	6.4	11.1	Positive for chromatid breaks in ns, negative for SCE	Jelmert *et al.* 1994
		MC, s	20	1.1	6.6	na		
		EC, s	46	0.9	6.9	na		
		Exp, ns	20	**1.5**	5.8	11.1		
		MC, ns	20	0.7	5.5	na		
		EC, ns	48	0.7	5.7	na		

Table 3. Cytogenetic surveillance studies among stainless steel welders using the manual metal arc method.

Key: *N*, number of subjects: Ab, aberrations; SS, stainless steel welders; MMA, manual metal arc method: MIG, metal inert gas method; na, not analysed; sd, standard deviation; crea, creatinine; Exp, exposed; IC, internal controls; MC, matched controls; s, smokers; ns, non smokers. Statistically significant differences are in bold.

effect markers in cytogenetic surveillance studies responded so weakly. This could be due to low levels of exposure with genotoxic effects too small to be detectable among study populations of up to 60 exposed individuals. Alternatively, are blood lymphocytes inappropriate as a surrogate tissue for the monitoring of genetic effects of chromium(VI) in humans?

Monitoring the biologically effective dose of chromium: DNA damage in blood lymphocytes

The few studies undertaken so far to monitor DNA damage in the blood lymphocytes of chromium(VI) exposed workers do provide an answer as to why cytogenetic markers respond so weakly to chromium(VI) exposure (Table 4).

As part of the cytogenetic surveillance study discussed earlier, Popp *et al.* (1991) have analysed the DNA from blood lymphocytes of stainless steel welders using filter elution techniques. The samples from welders showed reduced elution rates which the authors interpreted as being indicative of DNA–protein cross-links (DPC). Zhitkovich *et al.* (1996) have employed a potassium–SDS precipitation assay which provides more direct evidence for the existence of DPC. In a study of Bulgarian chromium platers they failed to observe significant differences in the DPC levels of blood lymphocyte DNA relative to controls not engaged in plating. Nevertheless, the levels of chromium in erythrocytes and urine of the platers confirmed that exposure to chromium(VI) had occurred.

Gao *et al.* (1994) have assessed oxidative DNA damage by monitoring the number of single strand breaks and modified DNA bases (8-hydroxydeoxyguanosine) in lymphocyte DNA from chromium(VI) production workers. There was no evidence for increased levels of oxidative DNA damage, although the workers' exposure to chromium could be verified using markers of internal exposure.

The studies by Gao *et al.* (1994) and Zhitkovitch *et al.* (1996) are particularly instructive because determinations of the chromium levels in blood lymphocytes were undertaken simultaneously with the measurement of DNA damage. The striking observation in both cases is that the differences in lymphocyte chromium levels between exposed and controls were not very pronounced, when at the same time all the other measures of internal exposure were markedly elevated in relation to controls. The absence of any pronounced

Study population	Level and duration of exposure	DNA damage: Group	N	Type of damage	Level of damage	Internal exposure	Author's conclusion	Reference
SS, MMA and MIG	na, 8 years	Exp IC	39 18	DPC DPC	0.78* 0.89*	U 18.9 μg g^{-1} crea na	Positive	Popp *et al.* 1991
Chromium (VI) production workers	1–55 μg m^{-3} 15 years	Exp	10	SSB 8-OHdG	59% breaks 0.037 8-OHdG/dG	U 5.97 μg g^{-1} crea P 2.8 μg l^{-1} WB 5.5 μg l^{-1} L 1.01 μg/10^{10} cells	Negative	Gao *et al.* 1994
		IC	10	SSB 8-OHdG	50% breaks 0.041 8-OHdG/dG	U 0.76 μg g^{-1} crea P 0.65 μg l^{-1} WB 0.73 μg l^{-1} L 0.76 μg/10^{10} cells		
Platers	0.5–130 μg m^{-3}	Exp	14	DPC (%)	1.53± 0.33	U 9 μg g^{-1} crea Ery 22.8 μg l^{-1} L 1.16 μg/10^{10} cells	Negative	Zhitkovich *et al.* 1996
				8-OHdG	1.45± 0.43	U 1.0 μg g^{-1} crea Ery 2.5 μg l^{-1} L 0.64 μg/10^{10} cells		

Table 4. DNA damage in blood lymphocytes of chromium-exposed workers.

Key: *N*, number of subjects; na, not analysed; Exp, exposed; IC, internal controls; EC, external controls; DPC, DNA–protein cross-links, U, urinary Cr; P, plasma Cr; WB, Cr in whole blood; Ery, Cr in erythrocytes; L, Cr in lymphocytes; crea, creatinine; SSB, DNA single strand breaks; 8-OHdG, 8-Hydroxydeoxyguanosine, dG, deoxyguanosine; * flow rates of DNA through membrane filters, low flow rate indicative of DPC.

differences in the lymphocyte chromium levels of exposed and controls, whatever the reason, could well be the factor which seriously limits the value of cytogenetic surveillance studies. Thus, lymphocytes may not be an appropriate surrogate tissue for the effect monitoring of chromium-exposed individuals.

Chromium levels in the lungs of chromium-exposed workers and of control populations

Another impeding factor in studies using blood lymphocytes is the toxicokinetics of inhaled chromium(VI). *Post-mortem* analyses of lung tissue obtained from chromium workers who died of lung cancer have established that chromium(VI) containing particles, once inhaled, stay in the lung for decades (Hyodo *et al.* 1980, Tsuneta *et al.* 1980). Even long after cessation of exposure most of the chromium is found in the respiratory tact whilst only small amounts reach liver and kidneys via the bloodstream.

From analyses of the organs of lung cancer patients with a history of employment in chromium(VI) production (Kishi *et al.* 1987), the amount of chromium residing in the lungs can be estimated as 30–70 mg, whereas the amounts found in livers and kidneys were 3.8 mg and 0.8 mg, respectively. In comparison, the lungs, livers and kidneys of deceased with no occupational chromium exposure contained 0.08–1.2 mg, or 0.3 mg and 0.07 mg, respectively. The lung chromium levels reported by Raithel *et al.* (1993) for stainless steel welders were lower than those found in chromium(VI) production workers (Table 5). Even so, the lungs of stainless steel welders showed concentrations of chromium 10–30 times higher than in unexposed individuals.

There is quite a large database documenting chromium levels in the lungs of occupationally unexposed referents (Table 5). Kollmeier *et al.* (1990) observed an age-dependent increase in lung chromium levels and found that men on average showed levels twice as high as women. These authors were even able to demonstrate differences due to environmental factors: the lung chromium contents of people living in a heavily industrialized conurbation (the Ruhr area) were significantly higher than those in individuals living in a city where occupations are mainly associated with trade and administrative services (Münster). Such differences are not detectable using any of the currently employed markers for internal doses of chromium.

The question arises as to how and where lymphocytes can come into contact with chromium(VI) when the bulk of inhaled chromium remains in the lung for very long periods of time. Two separate pathways can be envisaged: (1) lymphocytes, while travelling via the blood, take up chromium(VI) from the plasma that has leached from the lungs; (2) lymphocytes homing to the supporting tissues of the lungs come into contact with chromium-containing material and then migrate back to the blood stream.

Lymphocytes and chromium uptake

Lymphocytes continuously enter and leave lymphoid and non-lymphoid tissues via the blood. At any given time, about 2% of the total lymphocyte pool in the human body is in the blood. It is estimated that the residence time of lymphocytes in the blood is only about 30 min (Westermann and Pabst 1990), so that during 1 day approximately 500×10^9 lymphocytes travel through the blood, a number which is equivalent to the total lymphocyte population in the human body. However, not

Study population	Cr in lungs ($\mu g\ g^{-1}$ dry weight)	Reference
Chromium(VI) production, Case 1	397	Kishi *et al.* 1987
Case 2	1467	
SS welders	30–86	Raithel *et al.* 1993
Controls	0.31	Kishi *et al.* 1987
	1.37	Raithel *et al.* 1993
Referents, smokers	4.3	Pääkö *et al.* 1989
Referents, ex-smokers	4.8	
Referents, non-smokers	1.3	
Referents, industrial area	2.14	Kollmeier *et al.* 1990
Referents, non-industrial area	0.57	

Table 5. Chromium levels in the lungs of occupationally exposed and non-exposed individuals.

every single lymphocyte enters and leaves the blood during 1 day; the picture is further complicated by the fact that there are subsets which reside in certain tissues for long times without migrating through the blood. It is therefore unlikely that the lymphocytes in the blood are representative of the other lymphocytes distributed in the body.

Lymphocytes are produced in a variety of organs and are constantly being released to the blood. They can migrate through most of the organs of the body and have the ability to return to the blood. One established pathway of recirculation from organs to the blood is via the lymph nodes and the thoracic duct, accounting for approximately 5–10% of the lymphocyte population which returns to the blood each day. The spleen is by far the most important organ in lymphocyte recirculation, with 50% of all the recirculated lymphocytes being released by the spleen. In comparison, the daily recirculation of lymphocytes from the lungs back to the blood is negligible (Westermann and Pabst 1990).

Thus, only a very small fraction of the lymphocytes residing in the blood will have had the chance to take up chromium(VI) while travelling through the lungs. The vast majority of lymphocytes will have come into contact with chromium(VI) during their migration through the blood. These features are important when considering whether chromium-induced genotoxic effects occurring in lymphocytes are in any way predictive of the processes leading to mutations and eventually cancer in the respiratory tract. Given that the site of chromium(VI) uptake is outside the respiratory tract it is hard to see how genotoxic effects in lymphocytes can be predictive of similar events in lung cells. Furthermore, the toxicokinetics of inhaled chromium(VI) determine that cells of the respiratory tract are likely to be exposed to much higher amounts of chromium(VI) than lymphocytes. The chances that cancer-initiating events occur in these cells are orders of magnitude higher. The absence of any genotoxic effects in blood lymphocytes of exposed individuals cannot therefore be taken as an indication of absence of lung cancer risks.

The short blood transit time of lymphocytes is likely to be another complicating factor in the uptake of chromium(VI) by lymphocytes and might help explain the small differences in the lymphocyte chromium levels of exposed and unexposed individuals in the studies by Gao *et al.* (1994) and Zhitkovich *et al.* (1996).

Conclusions and perspectives

Chromium levels in body fluids, such as urine and blood plasma, are well accepted as markers of exposure to chromium in oxidation states (VI) and (III) and provide a measure of the internalized dose of chromium. However, on the basis of urinary or plasma chromium alone it is not possible to ascertain that exposure to chromium(VI) has occurred. Here, the measurement of chromium levels in erythrocytes provides a valuable adjunct since it is specific for exposure to chromium(VI), albeit at the expense of a lower sensitivity.

The relative lack of success of cytogenetic surveillance studies using blood lymphocytes—all the more surprising in view of the strong genotoxicity of chromium(VI)—can be explained in terms of the disproportionately small differences in lymphocyte chromium levels between exposed and controls. Furthermore, the toxicokinetics of inhaled chromium(VI) and the dynamics of lymphocyte traffic suggest that the processes giving rise to genotoxic effects in lymphocytes and to cancers of the respiratory tract are unrelated. Taken together, these considerations would suggest that blood lymphocytes are inappropriate for the monitoring of the biologically effective dose, and of early biological effects arising from exposure to chromium(VI) at levels below 50 $\mu g\ m^{-3}$, the current exposure limit in many industrialized countries. At higher exposure levels however, effect monitoring using lymphocytes may well be useful.

This rather sobering conclusion provokes the question whether there are alternative approaches to chromium biomonitoring which would be more meaningful in relation to lung carcinogenesis. As a first step in this direction it would be necessary to explore the relationship between urinary chromium and the amounts of chromium deposited in the lungs. This could perhaps be achieved if there were a record of the chromium levels in the urine of deceased whose lungs were analysed *post-mortem* for chromium. There is a great need to develop and evaluate non-invasive methods which would allow the monitoring of chromium levels in the lungs of exposed individuals. A possibility which is currently being actively pursued in our laboratory is the use of magnetic resonance imaging as a method for the monitoring of internalized doses of chromium(VI) in lung tissue.

Perhaps the biggest challenge in chromium biomonitoring is to bridge the gap between the monitoring of internalized doses and effect monitoring. Given the problems associated with utilizing lymphocytes as a surrogate tissue, effect monitoring ideally would have to concentrate on cells of the airways. However, this is complicated by the difficulty of access to these tissues and by the need to propagate such cells in culture. With the recent refinements in the use of the 'Comet assay' it should be possible to assess DNA damage in single *ex vivo* isolated cells.

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References

ANGERER, J., AMIN, W., HEINRICH-RAMM, R., SZADOWSKI, D. AND LEHNERT, G. (1987) Occupational chronic exposure to metals, I. Chromium exposure of stainless steel welders—biological monitoring. *International Archives of Occupational and Environmental Health*, **59**, 503–512.

ARAKI, S. AND AONO, H. (1989) Effects of water restriction and water loading on daily urinary excretion of heavy metals and organic substances in metal workers. *British Journal of Industrial Medicine*, **46**, 389–392.

ASHBY, J. AND RICHARDSON, C.R. (1985) Tabulation and assessment of 113 human surveillance cytogenetic studies conducted between 1965 and 1984. *Mutation Research*, **154**, 111–133.

BUKOWSKI, J.A., GOLDSTEIN, M.D., KORN, L.R. AND JOHNSON, B.B. (1991) Biological markers in chromium exposure assessment: confounding variables. *Archives of Environmental Health*, **46**, 230–236.

CHOI, Y.J., KIM, Y.W. AND CHA, C.W. (1987) A study on sister chromatid exchanges in lymphocytes in some metal plating workers. *Korea University Medical Journal*, **24**, 249–257.

GAO, M., LEVY, L.S., FAUX, S.P., AW, C.T., BRAITHWAITE, R.A. AND BROWN, S.S. (1994) The use of molecular epidemiological techniques in a pilot study on workers exposed to chromium. *Occupational and Environmental Medicine*, **51**, 663–668.

GRAY, J.S. AND STERLING, K. (1950) The tagging of red cells and plasma protein, with radioactive chromium. *Journal of Clinical Investigation*, **29**, 1604–1613.

GYLSETH, B., GUNDERSON, N. AND LANGARD, S. (1977) Evaluation of chromium exposure based on a simplified method for urinary chromium determination. *Scandinavian Journal of Work and Environment Health*, **3**, 28–31.

HUSGAFVEL-PURISAINEN, K., KALLIOMÄKI, P.L. AND SORSA, M. (1982) A chromosome study among stainless steel welders. *Journal of Occupational Medicine*, **24**, 762–766.

HYODO, K., SUZUKI, S., FURUYA, N. AND MESHIZUKA, K. (1980) An analysis of chromium, copper and zinc in organs of a chromate worker. *International Archives of Occupational and Environmental Health*, **46**, 141–150.

IARC (1990) Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 49, *Chromium, Nickel and Welding* (International Agency for Research on Cancer, Lyon).

JELMET, Ø., HANSTEEN, I.L. AND LANGÅRD, S. (1994) Chromosome damage in lymphocytes of stainless steel welders related to past and current exposure to manual metal arc welding fumes. *Mutation Research*, **320**, 223–233.

KALLIOMÄKI, P.L., RAHKONEN, E., VAARANEN, V., KALLIOMÄKI, K. AND AITTONIEMI, K. (1981) Lung-retained contaminants, urinary chromium and nickel among stainless steel welders. *International Archives of Occupational and Environmental Health*, **49**, 67–75.

KISHI, R., TARUMI, T., UCHINO, E. AND MIYAKE, H. (1987) Chromium content of organs of chromate workers with lung cancer. *American Journal of Industrial Medicine*, **11**, 67–74.

KNUDSEN, L.E., BOISEN, T., CHRISTENSEN, J.M., JELNES, J.E., JENSEN, G.E., JENSEN, J.C., LUNDGREN, K., LUNDSTEEN, C., PEDERSEN, B., WASSERMANN, K., WILHARDT, P., WULF, H.C. AND ZEBITZ, U. (1992) Biomonitoring of genotoxic exposure among stainless steel welders. *Mutation Research*, **279**, 129–143.

KOLLMEIER, H., SEEMANN, J.W., ROTHE, G., MÜLLER, K.M. AND WITTIG, P. (1990) Age, sex, and region adjusted concentrations of chromium and nickel in lung tissue. *British Journal of Industrial Medicine*, **47**, 682–687.

KORTENKAMP, A., BEYERSMANN, D. AND O'BRIEN, P. (1987) Uptake of chromium(III) complexes by erythrocytes. *Toxicological and Environmental Chemistry*, **14**, 23–32.

KOSHI, K., YAGAMI, T. AND NAKANISHI, Y. (1984) Cytogenetic analysis of peripheral blood lymphocytes from stainless steel welders. *Industrial Heath*, **22**, 305–318.

LEWALTER, J., KORALLUS, U., HARZDORF, C. AND WEIDEMANN, H. (1985) Chromium bond detection in isolated erythrocytes: a new principle of biological monitoring of exposure to hexavelant chromium. *International Archives of Occupational and Environmental Health*, **55**, 305–318.

LITTORIN, M., HOEGSTEDT, B., STROEMBAECK, B., KARLSSON, A., WELINDER, H., MITELMAN, F. AND SKERFVING, S. (1983) No cytogenetic effects in lymphocytes of stainless steel welders. *Scandinavian Journal of Work and Environmental Health*, **9**, 259–264.

MUTTI, A., CAVATORTA, A., PEDRONI, C., BORGI, A., GIAROLI, C. AND FRANCHINI, I. (1979) The role of chromium accumulation in the relationship between airborne and urinary chromium in welders. *International Archives of Occupational and Environmental Health*, **43**, 85–91.

NAGAYA, T. (1986) No increase in sister-chromatid exchange frequency in lymphocytes of chromium platers. *Mutation Research*, **170**, 129–132.

NAGAYA, T., ISHIKAWA, N., HATA, H. AND OTOBE, T. (1991) Sister-chromatid exchanges in lymphocytes from 12 chromium platers: a 5-year follow-up study. *Toxicology Letters*, **58**, 329–335.

NIOSH, NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (1977) *National Occupational Hazard Survey 1972–1974* (Cincinnati, USA).

PÄÄKÖ, P., KOKKONEN, P., ANTITILA, S. AND KALLIOMÄKI, P.L. (1989) Cadmium and chromium as markers of smoking in human lung tissue. *Environmental Research*, **49**, 197–207.

POPP, W., VAHRENHOLZ, C., SCHMIEDING, W., KREWET, E. AND NORPOTH, K. (1991) Investigations of the frequency of DNA strand breakage and cross-linking and of sister chromatid exchange in the lymphocytes of electric welders exposed to chromium- and nickel-containing fumes. *International Archives of Occupational and Environmental Health*, **63**, 115–120.

RAITHELL, H.J., SCHALLER, K.H., KRAUS, T. AND LEHNERT, G. (1993) Biomonitoring of nickel and chromium in human pulmonary tissue. *International Archives of Occupational and Environmental Health*, **65**, S197–S200.

SARTO, F., COMINATO, I., BIANCHI, V. AND LEVIS, A.G. (1982) Increased incidence of chromosomal aberrations and sister chromatid exchanges in workers exposed to chromic acid in electroplating factories. *Carcinogenesis*, **3**, 1011–1016.

SBRANA, I., CARETTO, S., LASCIALFARI, D., ROSSI, G., MARCHI, M. AND LOPRIENO, N. (1990) Chromosomal monitoring of chromium-exposed workers. *Mutation Research*, **242**, 305–312.

STRINDSKLEV, I.C., HEMMINGSEN, B., KARLSEN, J.T., SCHALLER, K.H., RAITHEL, H.J. AND LANGARD, S. (1993) Biologic monitoring of chromium and nickel among stainless steel welders using the manual metal arc method. *International Archives of Occupational and Environmental Health*, **65**, 209–219.

TOLA, S., KILPIÖ, J., VIRTAMO, M. AND HAAPA, K. (1977) Urinary chromium as an indicator of the exposure to welders on chromium. *Scandinavian Journal of Work Environment and Health*, **3**, 192–202.

TSUNETA, Y., OHSAKI, Y., KIMURA, K., MIKAMI, H., ABE, S. AND MURAO, M. (1980) Chromium content of lungs of chromate workers with lung cancer. *Thorax*, **35**, 204–297.

WESTERMANN, J. AND PABST, R. (1990) Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? *Immunology Today*, **11**, 406–410.

WOGAN, G. (1992) Molecular epidemiology in cancer risk assessment and prevention: Recent progress and avenues for future research. *Environmental Health Perspectives*, **98**, 167–178.

ZHITKOVICH, A., LUKANOVA, A., POPOV, T., TAIOLI, E., COHEN, H., COSTA, M. AND TONIOLO, P. (1996) DNA–protein crosslinks in peripheral lymphocytes of individuals exposed to hexavelant chromium compounds. *Biomarkers*, **1**, 86–93.

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I am pleased to be able to report to readers and authors that just before Christmas 1996, Taylor & Francis received notification that ***Biomarkers*** will now be listed in *Current Contents*. This is a significant achievement for a new journal to be listed so soon after launch. It will of course mean that it becomes a more widely known and read journal, with consequent increased exposure for papers. Thanks to the Editorial Board and authors and reviewers who have helped to make this happen.

Editor